IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants: Grigori N. Enikolopov and John Mignone

Application No.: 09/444,335 Group: 1632

Filed: November 19, 1999 Examiner: R. Schnizer

For: TRANSGENIC MICE EXPRESSING FLUORESCENT PROTEIN IN MULTIPOTENT STEM AND PROGENITOR CELLS

CERTIFICATE OF MAILING

I hereby certify that this correspondence is being deposited with the United States Postal Service with sufficient postage as First Class Mail in an envelope addressed to Assistant Commissioner for Patents, P.O. Box 2327, Arlington, VA 22202

on 8/2/02 Kathleen Riley
Date Signature

Kathleen Riley
Typed or printed name of person signing certificate

DRM 5/22/03

CM 5/22/03

DECLARATION OF ROBERT M. HOFFMAN, PH.D.

UNDER 37 C.F.R. § 1.132

Assistant Commissioner for Patents
P.O. Box 2327
Arlington, VA 22202

Sir:

I, Robert M. Hoffman, of La Jolla (P.O. Box 1296), California 92038-1296, declare and state that:

1. I obtained a Ph.D. in Biology from Harvard University, Cambridge, Massachusetts. My current position is Professor in the Department of Surgery at the University of California, San Diego, Medical Center. I am also President, Chairman of Board and Chief Executive Officer of AntiCancer, Inc., 7917 Ostrow Street, San Diego, California. I have published

about hundred and sixty peer-reviewed articles in the medical literature. A copy of my Curriculum Vitae and list of publications are attached as Exhibit A.

2. I have not been employed and I am not currently being employed by Cold Spring Harbor Laboratory.

3. I am not being compensated for providing this Declaration.

4. I am familiar with the contents of PCT Application WO 01/36482 (copy enclosed) which is a related PCT application of the referenced U.S. Application No.: 09/444,335, filed November 19, 1999, and with the following references:

> Zimmerman, L., *et al.*, "Independent Regulatory Elements in the Nestin Gene Direct Transgene Expression to Neural Stem Cells or Muscle Precursors," Neuron, Vol.12:11-24 (1994).
>
> Chiocchetti, A., *et al.*, "Green Fluorescent Protein As A Reporter of Gene Expression In Transgenic Mice," (Bichim. Biophys. Acta 1352(2): 193-202, (1997, May).

5. I am familiar with transgenic animals that express the *LacZ* reporter gene at the time the subject application was filed.

6. Over the last several years, I have directed research in the field of whole-body optical (fluorescence) imaging. Whole body optical imaging is a non-invasive imaging technique of large-scale structures in an animal's body and can detect fluorescence from green fluorescent protein (GFP) in an intact animal. For low-magnification, animals are illuminated within a fluorescence light box and directly viewed with a thermoelectrically cooled color charge-coupled device camera. Higher-magnification images are made with the camera focused through an epi-fluorescence dissecting microscope. Copies of several of my articles in the field of whole body fluorescence imaging are attached as Exhibit B. These articles were published during the years 2000 and 2001. In addition, patents issued to AntiCancer, Inc., San Diego, California, that relate to using the technique of whole body imaging to follow the progression of metastasis, are attached as Exhibit C.

To my knowledge, no live transgenic animal that employed green fluorescence protein as a reporter of gene expression was studied by whole body fluorescence imaging prior to November 19, 1999.

7. Based on my expertise, Dr. Enikolopov requested that I examine his transgenic mice. I agreed and Dr. Enikolopov provided me with young adults of his transgenic mice. It is my understanding that these mice were produced as described in PCT Application No. WO 01/36482, specifically as described with respect to Figs. 1A, 1B and 2.

8. I collaborated in experiments conducted to study the fluorescence pattern in the transgenic young adult mice that we had received form Dr. Enikolopov. These experiments are described below. Results are presented in Data Exhibits I-V.

Whole body optical imaging of green fluorescent protein, essentially as described by Yang, et al., PNAS, Vol. 97, No. 3, pp.1206-1211 (2000), was conducted in live young adult mice, that had been shaved. Using this technique, my collaborators and I were able to detect GFP expression in the retina and brain (Data Exhibit IA) of Dr. Enikolopov's transgenic mice, without further manipulation of the live, young adult mice.

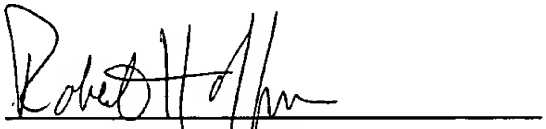
We also investigated the presence of GFP expression in the brain and in internal organs of Dr. Enikolopov's mice by employing the skin flap technique, described in Yang, et al., PNAS, Vol. 99, No. 6, pp. 3824-3829 (2002). Essentially, an arc-shaped incision was made in live, anesthetized mice and the skin and subcutaneous connective tissue was separated to free the skin flap. The skin flap can be opened repeatedly to image the fluorescent pattern of the live mouse. This technique results in reduced scatter of fluorescence photons. As seen in Data Exhibits IB-IE and II-V, we observed strong and well defined fluorescence in the brain, pancreas and testes of the transgenic young adult mice received from Dr. Enikolopov.

9. I was surprised to detect such strong, well defined fluorescence in a live, young adult transgenic animal that had, integrated into its genome, GFP as a reporter of nestin gene expression. I was also surprised by the presence of GFP under control of regulatory elements of the nestin gene in the pancreas and testes of these transgenic mice and by the intensity and definition of the fluorescence observed in these organs.

10. Prior to conducting the experiments described above, my collaborators and I had been successful in studying GFP models employing orthotopically implanted tumors, and in imaging the fluorescence of GFP-expressing bacteria from outside intact infected animals. We had also observed fluorescence from organs of nude or normal mice labeled by directly injecting a high viral load (i.e., 8×10^{10} plaque forming units/ml) of adenoviral GFP (DNA expression vector) into either the brain, liver, pancreas, prostate or bone marrow of the mice. (Our reports in the scientific literature are shown in Exhibit B).

However, I would not have expected that a transgenic mouse that had, integrated into its genomic DNA, the GFP gene, under the control of regulatory elements of the nestin gene, and that was produced by introducing into a fertilized egg, DNA that included GFP under the control of regulatory elements of the nestin gene, would exhibit the fluorescence intensity and fluorescence definition that we observed in our work with Dr. Enikolopov's mice.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.



Robert M. Hoffman, Ph.D.

July 29, 2002

Date